PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12P 21/08, C07K 15/28, A61K 39/00

A1

(11) International Publication Number: WO 91/10742

(43) International Publication Date: 25 July 1991 (25.07.91)

(21) International Application Number: PCT/US91/00319

(22) International Filing Date: 16 January 1991 (16.01.91)

(30) Priority data:
465,035 16 January 1990 (16.01.90) US

(71) Applicant: REPLGIEN CORPORATION [US/US]; One Kendall Square, Building 100, Cambridge, MA 02139 (US).

(72) Inventors: HIGGINS, Paul, J. ; 8 Sheridan Avenue, Medford, MA 02153 (US). SCOTT, Charles, F., Jr. ; 95 Gainsborough Street, Apt.406, Boston, MA 02115 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, One Financial Center, Suite 2500, Boston, MA 02111-2658 (US).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MONOCLONAL ANTIBODY SPECIFIC FOR NON-IMMUNODOMINANT EPITOPE OF HIV PROTEINS

(57) Abstract

An antibody capable of recognizing a group common determinant and a non-immunodominant epitope of the envelope protein of HIV, wherein the binding of the antibody to the envelope protein is not blocked by serum from an HIV-infected patient.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CS	Czechoslovakia
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GN	Guinea
GR	Greece
HU	Hungary
IT	Italy
JP	Japan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
MC	Monaco
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NL	Netherlands
NO	Norway
PL	Poland
RO	Romania
SD	Sudan
SE	Sweden
SN	Senegal
SU	Soviet Union
TD	Chad
TG	Togo
US	United States of America

MONOCLONAL ANTIBODY SPECIFIC FOR NON-IMMUNODOMINANT EPITOPE OF HIV PROTEINS

Background of the Invention

This invention relates to antibodies specific for Human Immunodeficiency Virus (HIV).

HIV is the proposed causative agent of Acquired Immune Deficiency Syndrome (AIDS). (Popovic et al., 1984, Science 224:497). It is a pathogenic human retrovirus whose genome is capable of encoding at least six gene products. The env gene encodes a 160 kDa glycosylated protein (gp160) that is processed by proteolysis into a 120 kD external glycoprotein (gp120) and a 41 kD transmembrane protein (gp41). gp120 is anchored to the virion by noncovalent interactions with gp41. gp120 and gp41 are present on the surface of both virion particles and virus-infected cells.

Different strains of HIV vary in the amino acid sequences of proteins encoded by the viral genome, particularly in the amino acid sequence of the external envelope glycoprotein gp120 (Starcich, 1986, Cell 45:637; Hahn et al., 1986, Science 232:1548). Over its entire length, the gp120 polypeptide sequence varies from one HIV variant to the next by approximately 20-25%. The extent of variation is not constant over the whole envelope protein. There is a pattern of conserved and variable regions, which suggests that the protein is divided into regions responsible for distinct functions. A number of different regions have been identified; for example, the CD4 binding domain, the principal neutralizing determinant, and cytotoxic T-cell recognition determinants.

The use of antibodies to target cells has previously been used in the treatment of cancer and other disorders. Zarling et al. (EPO 308 936) disclose

antibody heteroconjugates specific for the gp120 principle neutralizing domain which selectively kill HIV-infected cells. Pincus et al. (J. Immunol. (1989) 142;3070) describe antibody-toxin conjugates that also recognize an immunodominant region of gp120, and Till et al. (Proc. Nat. Aca. Sci., 1989, 86;1981) disclose anti-gp41-toxin conjugates.

Summary of the Invention

The invention features an antibody capable of recognizing a non-immunodominant epitope of the envelope protein of HIV, wherein the binding of the antibody to the envelope protein is not blocked by serum from an HIV-infected patient. As used herein, "antibody" refers to a whole antibody molecule, or to a fragment or to a modification of an antibody, e.g. a fragment of an antibody bay be the Fab_2 fragment of the molecule, the Fab^1 fragment , or the heavy or light chain alone; and a modification, for example, may be a linear polypeptide molecule which includes both the heavy and light chains, as described in Huston et al., WO 88/09344 and Ladner et al., WO 88/01649. As used herein, "non-immunodominant" epitope means an amino acid sequence within the natural conformation of a protein that is not significantly immunogenic; i.e., does not elicit an antibody response in at least 75% of human patients. An antibody directed toward a non-immunodominant region of the HIV envelope protein can, according to the invention, bind that region due to the absence or low level of potentially competitive circulating antibodies. In contrast, an antibody directed towards an immunodominant region of the envelope protein will be partially or completely blocked from binding the targeted envelope protein region due to the presence of antibodies in the patient due to the patient's natural immune response to HIV infection. The non-immunodominant envelope region may be within the

gp120 or the gp41 portion of the envelope protein. Non-immunodominance can be measured in vitro by binding the antibody to a target antigen in the presence of HIV-positive human serum; an antibody specific for a non-immunodominant epitope of the target antigen will demonstrate a comparable binding efficiency to the target antigen in the presence or absence of HIV positive serum. The binding efficiency of the antibody to the target antigen in the presence of HIV-positive serum is at least 80% of its binding efficiency in the absence of HIV-positive serum.

In preferred embodiments, the non-immunodominant epitope recognized by the antibody is group common. As used herein, "group common determinant" means an antigenic portion of a protein encoded by an HIV strain that is not specific for that strain only, but is present on at least one other HIV strain. Preferably, the antibody is capable of binding to the surface of HIV envelope glycoprotein expressing cells; is capable of recognizing the region of the envelope protein between amino acid residues 473 and 759, inclusive, according to the numbering convention of Ratner et al., 1985, Nature 313:277; and is capable of recognizing that portion of gp120 contained within the 473 through 759 amino acid region; one example of such an antibody is that produced by cell line A.T.C.C. No. HB 10321.

In other preferred embodiments, the antibody is covalently linked to a toxin to form a conjugate, and the conjugate is capable of killing HIV-infected cells in the presence of human HIV+ serum; killing may occur via internalization of the antibody-toxin conjugate by HIV-infected cells. The conjugate may be made at the protein level by chemically linking the antibody and the toxin molecule or at the DNA level, by cloning the DNA sequence

encoding the antibody and ligating it to a toxin-encoding DNA sequence, as described in Huston, supra.

As employed herein, the term "toxin" is used to include the commonly designated toxins such as poisonous lectins, ricin, abrin, modeccin, diphtheria toxin, pseudomonas exotoxin or preferably the toxic A chain portions thereof, as well as other toxic agents such as radioisotopes, cytotoxic and carcinostatic drugs. "Toxin" may also refer to combinations of the various toxins, which can be coupled to one antibody molecule thereby accommodating variable cytotoxicity.

In another preferred embodiment of the invention, the HIV-specific antibody is linked to a second antibody that is specific for an effector cell to form an antibody heteroconjugate (also known in the art as heteroaggregates or heteroantibodies). The anti-HIV antibody of the heteroconjugate binds to an HIV-infected cell, i.e., the target cell to be killed, while the anti-effector antibody of the heteroconjugate binds to an effector cell such as those found within the peripheral blood lymphocyte (PBL) population, e.g., cytotoxic T lymphocytes (also known as T cells), monocytes (in particular, macrophages), granulocytes, or large granular lymphocytes which include cells with natural killer activity or antibody-dependent cellular cytotoxic activity, with the result that the antibody components of the heteroconjugate bridge the effector and target cells and thus promote killing of the target cell by the cytotoxic effector cell.

HIV-infected patients can be treated by administering an amount of the antibody-toxin conjugate or an antibody heteroconjugate of the invention sufficient to kill HIV-infected cells. During active production of virus, the viral envelope protein is expressed on the surface of infected cells. By

selectively killing the cells in which the virus is replicating using an antibody conjugate that reacts with virus-specific cell-surface antigens, the infectious cycle of the virus may be interrupted.

One advantage of an antibody or antibody-toxin conjugate or an antibody heteroconjugate of the invention is the non-immunodominance of the HIV envelope glycoprotein epitope that the antibody is specific for. A consequence of non-immunodominance, or failure of the human immune system to mount a detectable immune response to an epitope, is that there are few, if any, circulating antibodies to that epitope; i.e., to a gp160 non-immunodominant epitope of the envelope protein in an non-immunodominant epitope of the envelope protein in an HIV-infected patient. Therefore, when an antibody-toxin conjugate or antibody heteroconjugate of the invention is used to treat an HIV-infected patient, it can selectively target HIV-infected cells without competing with circulating antibodies for the target non-immunodominant epitope.

Another advantage of certain antibodies, antibody-toxin conjugates, and antibody heteroconjugates of the invention are their ability to recognize a group common determinant of the HIV envelope glycoprotein. Group common determinants are portions of the envelope polypeptide that are essentially invariant among different HIV strains. Therefore, an antibody capable of recognizing a group common determinant can recognize gp160 from any strain of HIV. Treatment of an HIV-infected patient according to the invention will thus not be limited to any one strain of HIV, but will include all strains to which the target determinant is common.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We first briefly describe the drawings.

Drawings

Fig. 1 is an schematic illustration of the gp160 protein, showing the gp120, gp41, p121, and pENV9 regions.

Fig. 2 is a graph showing results of an ELISA in which the binding specificity of the 1C1 antibody to a target antigen was tested.

Figs. 3(a) - 3(d) are graphs showing results of ELISAs in which the binding specificity was determined for the 1C1 antibody or a control antibody in the presence of HIV positive serum.

Figs. 4(a) - 4(d) are graphs showing results of FACS analyses using the 1C1 antibody.

We now describe the preparation and use of antibodies of the invention.

Immunogens

Two immunogens were used to generate antibodies of the invention: gp160 (Repligen Corp., Cambridge, MA) and the envelope protein fragment extending from amino acid residue 473 through residue 759, denoted pENV9 (Ivanoff et al., U.S. Patent No. 4,861,707).

gp 160 was prepared for immunization by emulsification in complete Freund's adjuvant (CFA) according to standard techniques (Difco Labs, Grand Island, NY).

Production of Monoclonal Antibodies

Balb/cJ female mice (Jackson Labs., Bar Harbor, ME) were immunized intraperitoneally with 70μg per mouse of gp160/CFA. The mice were given a booster immunization of gp160 in an emulsification in incomplete Freund's adjuvant three weeks later. Mice were bled and the sera assayed for the presence of antibodies reactive with the immunogen. Mice showing a strong serological response

were given a final booster immunization of response were given a final booster immunization of pENV9 in soluble form five weeks following the first booster immunization and, 3 days later, spleen cells from these mice were fused at a ration of 5:1 with SP2/0 (A.T.C.C. No. CRL8287, A.T.C.C. No. CRL8006) myeloma cells incapable of secreting both heavy and light immunoglobulin chains (Kearney et al., J. Immunol., 1979, 123:1548), by standard procedures based on the method of Kohler and Milstein, Nature (1975) 256:495.

Supernatants from hybridomas which appeared 10-21 days after fusion were screened for production of antibodies reactive with the pENV9 protein fragment.

Each well of a 96-well Costar flat-bottom microtiter plate was coated with pENV9 by placing a fifty microliter aliquot of a PBS solution containing the protein fragment at a final concentration of 2-10 ug/ml in each well. The pENV9 solution was aspirated, the wells washed and replaced with PBS + 0.5% BSA and incubated for 2 hours. Following incubation, the wells were aspirated, washed, and 50 ul of hybridoma supernatant was added and incubated for 2 hours. Following incubation, the wells were washed 3 times with PBS, and then incubated for 1 hr. with 50 ul of an appropriate dilution of goat anti-mouse immunoglobulin conjugated with horseradish peroxidase (HRP, Boehringer Mannheim, West Germany). The wells were washed again 3 times with PBS and 100 ul of 1 mM ABTS (2,2 azino-bis (3-ethyl benzth, azoline 6-sulfonic acid) in 0.1M Na-Citrate, pH 4.2, to which a 1:1000 dilution of 30% H_2O_2 had been added), the substrate for HRP, was added to detect bound antibody. After 30 minutes, absorbance was measured at OD_{410} on a Dynatech spectrophotometric autoreader (Virginia).

Four hybridomas (1C1, 1H9, 2D7, and 2H8) that tested positive for binding to the first immunizing antigen, gp160, were tested for their ability to bind to the second immunizing antigen, pENV9, and to gp160 from another different HIV strain. One hybridoma clone, designated 1C1, which demonstrated reactivity with the pENV9 fragment by ELISA, produced antibody which was also capable of recognizing an envelope protein determinant that is present in more than one strain of HIV, i.e., a group common determinant, as described below.

The above four antibodies were tested in an ELISA for binding to either gp160 from the HIV-IIIB isolate or gp160 from the HIV-RF isolate. The results, presented in Table I below, show that the pENV9-specific 1C1 antibody binds to gp160 of both the IIIB and RF isolates, as well as to pENV9, whereas the remaining three antibodies do not recognize all three proteins.

Table I

clone	$gp160_{IIIB}$	$gp160_{RF}$	pENV9	BSA (control)
1C1	+	+	+	-
1H9	+	-	-	-
2D7	+	+	-	-
2H8	+	+	-	-

The isotype of the 1C1 clone was determined by the ELISA method to be IgG_{2a} using goat-anti-mouse HRP (Zymed Labs, San Francisco, CA) preparations which correspond to each of the major immunoglobulin isotypes. The 1C1 clone was subcloned and rescreened for the ability to bind to the Antigens described above. The 1C1 subclone was expanded by intraperitoneal injection into pristane primed Balb/c mice. Ascites fluid was recovered from the mice and the antibody was purified by Protein A affinity chromatography, as described below.

Amplification and Purification of Monoclonal Antibodies

Purified 1C1 antibody was prepared by injecting a hybridoma subclone that repeatedly tested positive by ELISA intraperitoneally into pristane-primed syngeneic mice. The ascites which developed were recovered two to three weeks after injection, and the antibody was purified as follows, and then dialyzed against PBS.

Ascites fluid containing IgG2a 1C1 antibody was diluted five-fold in 0.1 M Tris/3 M NaCl pH 8.9, bound to a Protein-A-Sepharose affinity column equilibrated with the same buffer, and then eluted from the column with 0.15 M NaCl, 0.1 M acetic acid, Ph 3.0. Following elution, the antibody was immediately neutralized by the addition of 1 M Na_2HCO_3.

Binding Specificity of the 1C1 Antibody

The 1C1 antibody was tested in the ELISA for binding with pENV9, gp120, and p121, in order to map the epitope to which 1C1 binds. p121 (Chang et al. U.S. Patent No. 4,774,175) is an 83 amino acid protein fragment spanning amino acids from approximately 566-648 within the gp41 portion of gp160, and is completely contained within the pENV9 sequence. (These regions of gp160 are schematically illustrated in Fig. 1.) p121 includes a major immunodominant epitope of the gp41 protein (Chang et al., U.S. Patent No. 4,724,175 and Wang et al., 1986, Proc. Nat. Aca. Sci. 83:6159). Fig. 2 shows the results of the ELISA assay. These results demonstrate 1C1 binds specifically to both pENV9 and gp120, but does not bind p121. Therefore, the 1C1 antibody binds to a region of pENV9 that is also present in gp120, but that is not contained within the p121 portion. In a control experiment, an antibody specific for gp41 (Epitope, Inc., Beaverton, OR) was tested for binding to either pENV9, p121, or gp120, showing that the anti-gp41 antibody bound to pENV9 and p121, but did not bind to gp120, as expected.

Binding of the 1C1 Antibody in the Presence of Human Serum

A therapeutically useful exogenous antibody specific for the HIV envelope protein must be able to bind HIV, or HIV-infected cells expressing the envelope protein, in the presence of competing circulating antibodies.

The 1C1 antibody was tested for its ability to bind to target antigen in the presence of serum from an HIV-infected patient, and compared with a control antibody known to bind to an immunodominant region of gp41. Figs. 3(a) - 3(d) show the results of ELISAs in which microtiter wells were coated with the capture antigen. The 1C1 or control antibodies were then added in 50 ul of (1) undiluted HIV-negative serum, (2) undiluted HIV-positive serum, or (3) 0.5% BSA. After 2 hrs., the wells were washed and secondary antibody (sheep anti-mouse-HRP) which did not cross-react with human Ig was added. After 1 hr., the secondary antibody was removed, the wells were washed, ABTS was added, and the OD_{410} was measured after 30 min.

In Fig. 3(a), the 1C1 antibody was assayed for binding to the capture antigen, pENV9. The results demonstrate that 1C1 bound with almost equal efficiency to pENV9 in the presence of either HIV-positive serum or HIV-negative serum (Fig. 3a). Although the 1C1 antibody bound with the highest efficiency to pENV9 in the presence of HIV negative serum at antibody concentrations above 0.01 μg/ml, the binding of this antibody to pENV9 in the presence of HIV positive serum was about 88% as efficient as binding to pENV9 in the presence of HIV negative serum at an antibody concentration of 1 μg/ml, and over 95% as efficient at 10 μg/ml. Similar results were obtained when 1C1 binding was tested in the presence of HIV positive sera from four other patients. These

results demonstrate that, if there are pENV9-specific antibodies present in HIV positive serum, they are present in low enough titers or have a weak binding affinity or react with different pENV9 epitope so that they do not significantly interfere with the binding of the 1C1 antibody to pENV9. Thus, 1C1 is potentially useful as a therapeutic agent.

In Fig. 3(b), the ability of the 1C1 antibody to bind the p121 protein was tested. The 1C1 antibody did not bind p121 at all. (The minimal reactivity observed with p121 at 1C1 concentrations above 0.1μ/ml in 0.5% BSA was probably due to nonspecific binding.)

Two additional control experiments were performed using a control antibody, anti-gp41 (Epitope, Inc.) and tow target antigens, p121 and pENV9. Results present in Figs. 3(c) and 3(d) show that anti-gp41 binds to both target antigens, but binds more efficiently to p121 than to pENV9, at antibody concentrations above 0.1 μg/ml. The results also show that, at these same concentrations of antibody, the binding of anti-gp41 antibody is partially blocked by HIV positive serum. These results indicate that there are antibodies specific for p121 and pENV9 present in HIV positive serum that are capable of partially blocking the binding of the anti-gp41 antibody.

Binding of 1C1 Antibody to Cells

Whether the 1C1 monoclonal antibody binds to the surface of cells expressing the HIV envelope glycoprotein was determined by indirect immunofluorescence and analysis by FACs (Fluorescence Activated Cell Sorter, Methods in Enzymology, 1984, Parks et al., 108:197), as follows.

The 1C1 antibody was bound to either CV1 cells (A.T.C.C. No. CCL70) infected with a Vaccinia Virus recombinant containing the HIV env gene, which express both gp120 and gp41 on their surface (CV1-Env), or CV1

cells infected with a Vaccinia Virus recombinant that does not contain the HIV env gene (CV1-Lac), as a negative control. Construction of a recombinant capable of expressing the full-length HIV envelope gene is described in EPO 243 029, hereby incorporated by reference. Figs. 4(a) - 4(d) show these results.

In Fig. 4(a), the 1C1 antibody was bound to CV1-Env cells. If Fig. 4(a) is superimposed on Fig. 4(b), which shows a FACS profile for CV1-Lac cells incubated with the 1C1 antibody, there is a rightward shift (i.e., an increase) in fluorescence intensity in the CV1-Env cells compared to the CV1-lac cells, indicating that the 1C1 antibody binds HIV envelope glycoprotein expressing cells significantly better than the cells not expressing the envelope protein. This result is significant because it demonstrates that 1C1 binds to the target antigen in its native state, and suggests that cells expressing HIV envelope glycoprotein may be specific targets for an immunotoxin conjugate composed of 1C1 linked to a toxin. Figs. 4(c) and 4(d) are controls in which the 1C1 antibody was bound to uninfected CV1 cells and in which buffer alone was bound to CV1-Env Cells, respectively. (The background fluorescence that is apparent in the FACS analyses of CV1-Env and CV1-Lac cells is probably due to alterations in the cell membrane resulting from expression of foreign viral proteins.)

Preparation of Antibody Conjugates of Heteroconjugates

The antibodies may be conjugated to cytotoxic agents and used as immunotoxins (as described in, e.g., Vitetta et al., 1987, Science 238: 1098), or incorporated onto the surface of liposomes containing anti-HIV drugs or toxins to specifically target such drugs or toxins to HIV-infected cells. As employed herein, the term immunotoxin refers to a conjugate of an antibody with one or more toxins. Where combinations of

various toxins are coupled to one antibody molecule, coupling may occur by different chemical mechanisms; for example, covalent binding, affinity binding, intercalation, coordinate binding and complexation. The preferred coupling of the antibody to the toxin is, however, covalent binding either by chemical or genetic fusions.

In a preferred embodiment, the immunotoxin comprises an antibody reactive with a non-immunodominant, group common epitope of the HIV envelope protein linked to the exotoxin form Pseudomonas aeruginosa. Pseudomonas exotoxin (PE) is particularly preferably to other toxins because large amounts are easily prepared, because humans do not usually have neutralizing antibodies against it, and because it does not need to be separated into subunits before being conjugated. PE is an extremely active monomeric protein (molecular weight 66kD), secreted by P. aeruginosa which inhibits protein synthesis in eukaryotic cells. A preferred form of PE is the truncated molecule, designated PE40, from which the cellular binding domain has been removed (Pastan et al. EP Publication No. 0 261 671). PE40 can be linked to an antibody of the invention by chemical coupling, for example, using the heterobifunctional cross-linker SPDP (N-succinimidyl-3-(2-pyridyldithiol) propionate) Sigma, St. Louis, MO) (Pastan et al, 1986, Cell 47:641), or by genetic fusion (Chaudhary et al.,) 1989, Nature 339:394). Where an antibody heteroconjugate is preferred, any suitable method of conjugation of the antibodies may be used; for example, preferred method involves cross-linking the antibodies using the cross-linker SPDP according to the method of Karpovsky et al. (1984, J. Exp. Med. 160:1686). Following cross-linking, the heteroconjugates are separated from fee antibody by size exclusion chromatography.

An antibody-toxin conjugate or heteroconjugate of the invention can be tested for target specificity and efficiency by killing by incubating the conjugate with HIV chronically-infected cells that express HIV envelope glycoprotein or with uninfected cells, and pulsing the cells with ^{3}H-Thymidine of ^{14}C-Leucine. Toxicity can be measured by a decrease in cell division or protein synthesis in the infected cells relative to the uninfected control cells. The efficiency of cell killing can be calculated using a clonogenic assay, in which infected cells are incubated with a conjugate of the invention, plated by limiting dilution (Tarwell, 1981, J. Immunol. 126.1614), and the number of surviving cells is compared to identically treated uninfected cells.

Use

In a typical treatment employing antibodies of the invention as immunotoxins, the antibody (which binds to a protein that is expressed only in HIV-infected cells) is conjugated to a toxin (e.g. pseudomonas exotoxin) that is toxic to the HIV-infected cells (and to non-infected cells as well). By coupling the cytotoxic agent to the antibody, a high level of toxic efficacy can be achieved specifically against the target cell with a markedly low level of non-specific toxicity. The use of the toxic agent is possible because the antibody to which the agent is coupled will carry the agent specifically to the target (in this case, HIV-infected cells), thereby sparing non-infected cells from the toxin. Techniques that may be employed to conjugate antibodies to cytotoxic agents are described in detail in Vitetta et al, supra, and in European Patent Application No. 279,668, published August 24, 1988.

The antibodies of the invention can be incorporated into conventional pharmaceutical formulations for use in treating individuals that are

infected with HIV. In addition, such formulations may comprise pharmaceutically-acceptable carriers, diluents, salts and other materials well-known in the art. Isotonic saline, sterile water, 10% maltose, human serum albumin, glycine or other pharmaceutically-acceptable material may be used as diluents, carriers or solvents in preparing the pharmaceutical formulations comprising antibodies of the invention.

The pharmaceutical compositions may be in a variety of dosage forms which include solid, semi-solid and liquid forms as powders, pills, tablets, liquid solutions or suspension, suppositories, polymeric microcapsule, liposomes or injectable or infusible substances. The pharmaceutical formulations may be administered using conventional methods which include, but are not limited to, intravenous, oral, subcutaneous, intraperitoneal or intralymphatic. In addition, the antibody, immunotoxin or heteroconjugates of the invention may be administered in conjunction with other treatments to augment the effectiveness of the treatment.

Other Embodiments

Other embodiments are within the following claims. For example, since, for the most part, monoclonal antibodies are produced in species other than humans, they are often immunogenic to humans. In order to successfully use these monoclonal antibodies in the treatment of humans, it may be necessary to create a chimeric antibody molecule wherein the portion of the polypeptide involved with ligand binding (the variable region) is derived from one species and the portion involved with providing structural stability and other biological functions (the constant region) is derived from a human antibody. Methods for producing chimeric antibodies in which the variable domain is derived from one host and the constant domain is derived from a second

host are disclosed by Neuberger et al. (WO Publication No. 86/01533) and Morrison et al. (EP Publication No. 0 173 494), hereby incorporated by reference.

An alternative method, in which an antibody is produced by replacing only the complementarity determining regions (CDRs) of the variable region with the CDRs from an immunoglobulin of the desired antigenic specificity, is described by Winter (GB Publication No.) 2 188 638). For example, the CDRs of a pENV9-specific, murine monoclonal antibody which recognizes a group common determinant and a nonimmunodominant domain can be grafted onto the framework of a human antibody by recombinant DNA techniques. This arrangement is particularly beneficial for use in the therapeutic applications of monoclonal antibodies.

Deposit

Cell line 1C1-1H5 was deposited in the American Type Culture Collection on January 10, 1990, and assigned Accession Number HB 10321.

Applicants' assignee, Repligen Corporation, represents that the A.T.C.C. is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon and granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. 1.15 and 35 U.S.C. 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent,

whichever period is longer. Applicants' assignee acknowledges its duty to replace the deposit should the depository by unable to furnish a sample when requested due to the condition of the deposit. A copy of the A.T.C.C. Budapest Treaty deposit receipt will be furnished upon request.

Claims

1. An antibody capable of recognizing non-immunodominant epitope of the envelope protein of HIV, wherein the binding of said antibody to said envelope protein is not blocked by serum from an HIV-infected patient.

2. The antibody of claim 1, wherein said epitope is group common.

3. The antibody of claim 2 wherein said antibody is capable of binding to the surface of HIV envelope glycoprotein-expressing cells.

4. The antibody of claim 3 wherein said antibody recognizes the region of the HIV envelope protein between amino acid residues 473 and 759, inclusive.

5. The antibody of claim 4 wherein said antibody recognizes a portion of gp120 within said region.

6. The antibody of claim 5, said antibody being produced by cell line having A.T.C.C. No. HB 10321.

7. The antibody of any claims 1-6 further comprising a toxin covalently linked to said antibody to form a conjugate which is capable of killing HIV-infected cells in the presence of human HIV+ serum.

8. The antibody-toxin conjugate of claim 7 wherein said conjugate is capable of being internalized by HIV-infected cells.

9. The antibody of any claim 1-6 further comprising a second antibody covalently linked to said antibody to form a heteroconjugate.

10. A method of treating HIV-infected patients, comprising administering an amount of the antibody-toxin conjugate of claim 6 or the antibody heteroconjugate of claim 8 sufficient to kill HIV-infected cells.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/00319

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C12P 21/08; C07K 15/28; A61K 39/00

U.S. Cl.: 530/387,389;424/85.91,86

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/387,389 ; 424/85.91,86

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US.A 4,340,535 (VOISIN ET AL) 20 JULY 1982, see abstract.	6-10
Y	Gene, Volume 52, issued 1987, A SRINIVASAN ET AL, "Molecular characterization of human immunodeficiency virus from Zaire: nucleotide sequence analysis identifies conserved and variable domains in the envelope gene", pages 71-82, see page 78, figure 5 and page 80.	1-10
Y	Cell, Volume 46, issued 1986, J.M. COFFIN, "Genetic Variation in AIDS viruses", pages 1-4, see paragraph bridging pages 1 and page 2, and page 3.	1-10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [1]

15 April 1991

Date of Mailing of this International Search Report [2]

24 MAY 1991

International Searching Authority [3]

ISA/US

Signature of Authorized Officer [4]

Christine M. Nucker

Form PCT/ISA/210 (second sheet) (rev. 11-87)